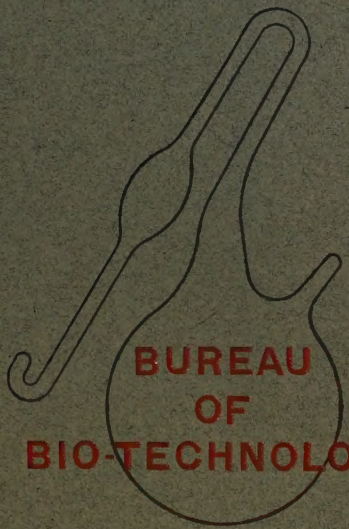
BULLETIN No. 6

AUGUST, 1922



CONTRIBUTIONS FROM THE BIOLOGICAL, CHEMICAL AND PHYSICAL RESEARCH LABORATORIES OF

MURPHY & SON, LTD.

LONDON, LEEDS AND NOTTINGHAM

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FOREWORD

AS indicated by the Table of Contents, this issue of the *Bulletin* contains contributions on subjects of interest and importance in several branches of industry. With reference to some of them, it may be said that the increasing number of technical purposes for which Fluorides are being used, and the difficulties encountered in the detection and estimation of minute quantities of these salts, render the appearance of our article on that subject opportune. The condition of Milk, Beer, Bread, etc., known as "ropiness," a defect due to biological causes, has long been a source of trouble in the dairying, brewing and baking industries, but it is erratic in its occurrence and very little is known with regard to the biological, physiological, or chemical factors which influence it. We believe our preliminary studies of the question in its relation to beer constitute a step of definite progress in our knowledge of the subject. Our series of papers on "Micro-organisms in the Leather Industries" is continued herein, and in future articles it is proposed to deal with the species of Aspergillus Mycoderma, etc., in the same way as that followed in discussing the species of Penicillium.

It must be evident to readers that the publication of our Laboratory results in this form involves expense not nearly met by subscriptions and advertising dues. We should like to increase, rather than curtail the circulation of the *Bulletin*, and Subscribers will assist us in our efforts by communicating their requirements to advertisers, mentioning the source of their enquiry.

MURPHY & SON, LTD.

August, 1922.

Micro-Organisms
in the
Leather Industries

3. SPECIES OF THE GENUS PENICILLIUM AND THEIR IDENTIFICATION—*Contd.*

OF the four species of Penicillium already named (Micro-Organisms in the Leather Industries, 2, Bull. Bur. Bio-Tech. 4, p. 88), only two of them, *P. expansum* and *P. decumbens*, occur at all commonly on leather, and the latter organism is the one most frequently met with. The separation of these two species is considerably simplified by the fact that they fall one each into the two sections, Eupenicillium and Aspergilloides, respectively, illustrated *loc. cit.* p. 90. After a little experience, a hand-lens examination, or even the colour and texture of surface growth will enable an observer to differentiate between the two species.

As these papers are intended for students and others not already expert in the identification of moulds, or even in mycological methods, some brief notes on the simple procedure to be adopted may serve a useful purpose. A preliminary microscopical examination of a mildewed spot, without disturbance, and uncovered, should be made with a low power, using a 1″ or $\frac{3}{4}$″ objective. This will enable the observer to form some opinion with regard to the nature of the organism, whether it is a species of *Penicillium*, *Aspergillus*, one of the *Mucoraceæ*, or none of these. This is important because the method of mounting for subsequent examination depends upon the nature of the organism to be dealt with; it also creates an impression of the gross appearance of the causative fungus, which will often assist recognition by means of a hand-lens when a microscope may not be available.

All the micro-fungi present difficulties in their preparation for critical examination, and the choice of the medium to be employed

is of considerable importance. In the case of most of the "moulds" it is useless attempting to mount in water. Immediately upon coming into contact with that liquid the conidia are detached from the conidiaphore and become aggregated in masses; the conidiaphores refuse to separate out, each filament retains a surrounding film of air, and the preparation becomes an uninterpretable mass of fungus elements and air bubbles. Lack of care in mounting is a source of much discouragement to students of the micro-fungi. Acetic acid and alcohol have been widely recommended for the purpose and they overcome the difficulty to some extent; the former may be used, but the strongly dehydrating action of alcohol is always operative on delicate fungus filaments and spores, and it should not be used where measurements are involved. For the technical examination of "moulds" generally, with the exception of all species belonging to the *Mucoraceæ*, the author uses a medium compounded as follows:—

Phenol (Pure crystals)	2 parts
Glycerine (Pure)	1 part
Lactic acid (Sp. Gr. 1·50) ...	2 parts
Distilled water	2 parts

The phenol is liquefied by gentle heating, after which the mixture may be made up in parts by volume.

The advantages possessed by this medium are that the conidia of *Penicillium* and *Aspergillus* species are retained in their natural positions to a much greater extent than is the case in either acetic acid or alcohol; there is no collapse of the hyphæ, nor shrinkage of conidia, and its refractive properties render staining unnecessary. It does not evaporate, and when used in the correct volume to spread to the edge of the cover glass only, a mount is prepared, which, for all practical purposes, is permanent without further attention. Its high viscosity permits the use of an oil immersion lens without undue lift or depression of the cover-glass when focussing.

For further examination of the fungus with higher powers, a specimen may be removed from the leather by the aid of a stiff platinum needle or the point of a scalpel. Care should be taken

to secure the trailing hyphal filaments which bear the conidiaphores with as little disturbance of the surface as possible. The specimen should be transferred to one drop of the medium already placed on a slide, and carefully teased out until no opaque particles remain. A cover-glass is then lowered obliquely from one edge of the drop, care being taken to avoid the inclusion of air bubbles.

In the examination of Penicillium species, the morphological features of the conidiaphore call for special observation. It may be short or long, simple or branched ; it may consist, normally, of a single filament, or under some conditions of development, particularly in the case of *P. expansum*, a number of filaments may cohere, forming a compact, stalk-like fascicle known as a *coremium*. In some species the outer wall is smooth, in others, finely granular or minutely warted, and it needs careful examination to ascertain the true character in this respect. The conidial apparatus may consist of the metulæ and the sterigmata, surmounted by the conidia (Eupenicillium), or the metulæ may be absent, the sterigmata being attached directly to the tip of a filament (Aspergilloides) ; these elements, also, may be smooth or granular, and their dimensions are of diagnostic value. The conidia may vary in different species, from elliptical to globose in shape, and from 2μ to 8μ in length ; they may be smooth, or delicately warted, and observation on this point must be made with a 1/12″ (or 2 mm.) objective. With regard to colour, conidia which appear green in mass may not exhibit colour under a high power ; microscopically, they may be colourless, green, olive or brown. In the early stages of development the conidia are always colourless.

With attention to the various points noted above, together with the descriptions which follow, it will be possible to determine with certainty any of the four species so far found on leather without having to resort to culture methods.

In case of the occurrence of a species which cannot be correlated with any one of the four, it will be necessary to make an extended cultural examination on various media, but it is presumed that this will be attempted only by those with some experience in the preparation of such media, and in the technique of pure culture.

For certain sorting tests, Thom (*Bur. Animal Ind.*, Bull. 118, p. 94-5) makes use of the following four media :—

1. 15 per cent. gelatine in distilled water.
2. 15 per cent. gelatine in distilled water, plus 3 per cent. cane sugar.
3. Bean (or potato) decoction, plus 1·5 per cent. agar.
4. Bean (or potato) decoction agar, plus 3 per cent. cane sugar.

Portions of each of these are transferred to Petri dishes and inoculated with conidia of the fungus to be determined. Nos. 1 and 2 are used for evidence of liquefaction, and 2 and 3 for coremium and sclerotium formation. The same worker also makes use of other media, particularly Dox's solution, for which he gives the formula, as follows :—

Distilled water	3,000 c.cs.
Magnesium sulphate	1·5 grams.
Dipotassium phosphate (K_2HPO_4) ...	3·0 grams.
Potassium chloride	1·5 grams.
Ferrous sulphate	0·03 gram.

This medium is unaffected by sterilization, and, as it contains neither carbon nor nitrogen, it can be made the basis for cultural work in testing the availability of these elements in various forms.

The writer has found that malt-wort agar gives normal development comparable with results obtained on the bean and potato agars of Thom, while the same medium produces characteristic growths, quite in agreement with those recorded by Westling on prune-juice media. Therefore, for all ordinary purposes of identification, the two media, consisting of 15 per cent. gelatine in distilled water, and malt-wort agar, respectively, will give ample data for determination. For further cultural work, Dox's fluid medium should be employed, adding such carbohydrates, glucosides, tannins or other carbon compounds as experiment may require.

For experiments on the availability of pyrogallol and catechol as sources of carbon, the writer has used extracts of valonia and quebracho respectively.

Attention should be directed to the macroscopical appearances of different species on artificial media, which often differ from

those presented by the same species growing naturally on such substrata as leather, bark, etc. The physiological effects produced during development should be noted, as they are frequently of diagnostic value ; indeed, the species of Penicillium could not be successfully separated on morphological considerations alone.

The colour of the surface growth, termed by Westling the "conidial herbage," should be noted throughout its various stages of development ; there is usually a definite sequence of changes in the colours seen in a young, and in an aged culture. The reverse side of a colony may be white, grey, brown, yellow or red, and the production of such colour is a physiological constant for each species. The texture of the surface growth may be velvety, floccose, or granular ; it may, or may not, produce long aerial hyphæ. The odour is usually characteristic and it may be pleasant, musty, ammoniacal, etc. Transpiration, with the production of drops of liquid, which lie as minute dewdrops on the surface of the culture, occurs in some instances. This is a feature of both *P. decumbens* and *P. lanosum* among the leather species. Information with regard to the liquefying action of different species on gelatine is important, and among the four about to be described, *P. decumbens* is characterised, and, in fact, differentiated by the absence of a proteolytic enzyme that will liquefy gelatine. Some species develop acidity, others alkalinity, and the reaction of a culture may be determined by means of litmus, but at present, very little value can be attached to information obtained in this way.

Descriptions of the four species found on leather, or tanning materials, now follow, and for further information with regard to purely cultural details reference should be made to the papers of Thom and Westling already cited.

CULTURAL AND PHYSIOLOGICAL FEATURES.

P. expansum (Link) Thom (*Bur. Animal Ind.*, Bull. 118, 1910, p. 27, fig. 1).

Occurrence in nature : Is found constantly on pomaceous fruits and is nearly always present on stored apples. It also occurs frequently on stale flour-paste, and on cereal and

fruit substances in the larder, although, it is not the common blue mould of cheeses. It is one of the most cosmopolitan of the fungi, and Petri dish exposures to the atmosphere of towns usually produce one or more colonies.

On leather : It has been found much more frequently on old, de-natured leather, such as worn-out boots and leather scraps, than on newly-tanned goods, and, so far as the author's experience goes, it has not been met with on skin, limed pelts, or fancy leathers. On old leather it occurs usually in spots, or circular patches, up to 1 cm. diameter, more or less concentrically zoned. It may be recognised by its bright blue colour, darkest in the centre, white at the edges of the spots, with a clear-blue median zone. It presents a slightly velvet appearance on leather, increasing in coarseness towards the centre, as distinct from the

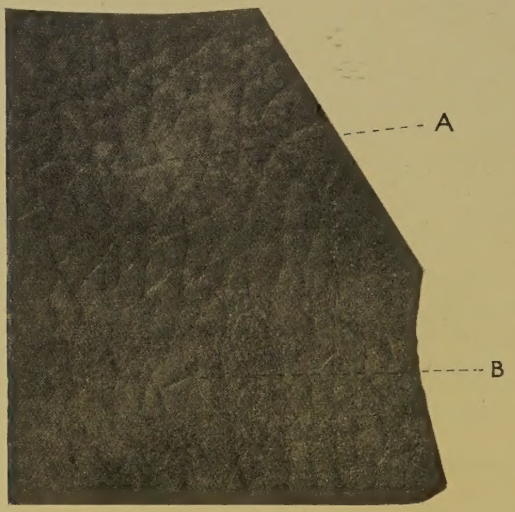

FIG. 12.
HARNESS LEATHER INOCULATED WITH (A) *Penicillium expansum*, AND (B) *P. decumbens*.

floccose habit which is characteristic of this species on culture media. In comparative experiments on heavily stuffed belting hide and a fine quality harness leather by direct inoculation, it was found to develop only on the latter and then very slowly in small, closely restricted areas, Fig. 12, A.

On tanning materials : This species was found in small proportion among moulds isolated from tan liquors. It has not been found in the dust of valonia or of quebracho, although, it develops slowly in the tanning extracts prepared from each of them.

On wort agar : Grows rapidly at 20°C ; white at first, then pale blue-green to darker blue-green, always with a white edge during growth. In a tube or other vessel limiting growth, the colour extends to the edge of the culture and the whole surface finally becomes grey-green. In young and growing cultures it is always floccose (downy or woolly), while, in old cultures, this feature may disappear owing to the production of masses of conidia which form a thick uniform layer. It is the type species of the section Eupenicillium, with well-marked branching conidiaphores and penicillate fructifications. It is specially characterised by the production of a coremium form. The latter consists of a bundle of conidiaphores, as already described, surmounted by a dense, green head of conidia, the whole having the appearance of a miniature drum-stick, 2 mm. high. The odour in mass is distinctly fruity. Underside of colony yellowish brown, soon wrinkled.

On 15 per cent. gelatine : Grows much less vigorously than on above medium ; surface growth of bluish green to green colour ; produces liquefaction in about two weeks.

P. decumbens Thom (*Bur. Animal Ind.*, Bull. 118, 1910, p. 71, fig. 28).

Occurrence in nature : Very little information is available with regard to the general occurrence of this species and Thom does not refer to the substratum on which his type specimen

was found. Its occurrence on leather and tanning materials was first reported by the writer in 1921 (Bull. Bur. Bio-Tech., No. 4, and *Leather Trades' Year Book*, 1921, p. 106), since when its constant presence on these materials has received ample confirmation.

On leather : It has been found on all kinds of leather, including both vegetable and chrome tanned goods ; on limed pelts, and on sheep-skins from the soaks. In inoculation experiments it has always grown vigorously, and equally well on the grain, or on the flesh side. In some cases it has been observed that growth commences at the edge of the leather, and for a time is confined to the area lying between the grain-layer and the corium. In one experiment with a sample of heavily-stuffed hide, the fungus developed all round the four edges before extending to the grain-layer, which, finally, it covered completely with a thick, dark-green deposit of conidia. Figs. 13, 14, and 15 illustrate this experiment. Fig. 16 shows the appearance presented by the tufts of conidia composing the surface growth, as seen under the microscope, using a 2/3″ objective. It is a characteristic feature of this species to spread rapidly, in rather large, effused, indeterminate colonies, which

FIG. 13.
P. decumbens ON EDGE OF HIDE.

completely obliterate the grain, and this condition is well seen in Fig. 15. On soaked sheep-skin, however, it invariably commences with bluish-green spots at a great number of points before becoming characteristic.

On all leathers its appearance varies according to age; at first bluish-green, it appears to be a stain, rather than a surface growth. It afterwards exhibits a slightly woolly appearance, becoming greyish-green. Finally, it presents a dark-green velvety appearance, and under a low power the long curling verticils, composed of chains of strongly adhering conidia, may be seen as shewn in Fig. 16. Its colour, in different stages of its growth on leather, is always uniform, and the colonies do not develop a white periphery, as in the case of *P. expansum*. It does not possess any marked odour, and in the experiment with stuffed hide, to which reference has already been made, no odour beyond that of the leather could be detected in its worst stage.

There is no doubt that this species has been frequently recorded from leather under the name *P. glaucum*. Mr. Alfred Seymour-Jones ("Collegium," No. 528, 1914, p. 206) has reported a case of damage to harness leather by a species of Penicillium, and has described certain "pips" due to the

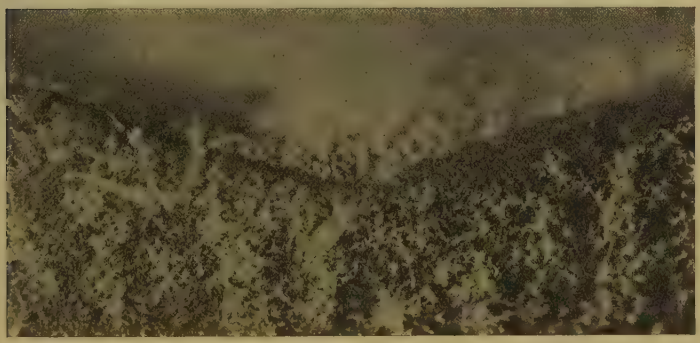

FIG. 14.
P. decumbens SPREADING TO SURFACE OF HIDE.

effects of fungal development on the grain. In the same paper, which is one of a few serious contributions to this aspect of leather technology, the relationship of the physiological structure of the skin to the mode of infection by moulds is discussed, and methods for prevention and remedial treatment are fully described. By the courtesy of Mr. Seymour-Jones, through Mr. P. Hampshire, of this Bureau, the writer has had the opportunity of examining the sample of leather used in illustration of the article referred to above. Careful examination of this sample led to the belief that conidia of the organism responsible for the original damage had not survived, as no growth could be obtained from the " pips " themselves. On the other hand, in an experiment to determine the nature of any organisms that might develop from conidia, either derived from the " pips," or occurring sporadically on the surface of the leather, a profuse and typical growth of *P. decumbens* was obtained ; the same fungus appeared on a piece of similar leather from another source, used as a control in this experiment. It was

FIG. 15.
OBLITERATION OF GRAIN BY *P. decumbens* ON HIDE.

inevitable, perhaps, at the time of Mr. Seymour-Jones' investigation, that the mould should have been identified as *P. glaucum*.

In another experiment in which a piece of hide was immersed in water, a filamentous growth of fungal hyphæ, 1 to 2 cms. in length, was produced at right angles to the leather; this was a totally-submerged growth, which was altogether unlike a *Penicillium*, and rather suggested the vegetative hyphæ of some species belonging to the *Mucoraceæ*. It was transferred to a Petri dish for further examination, where it produced a pure culture of *P. decumbens*.

It is not possible to discuss here methods for preventing the development of this or other species of Pencillium on leather; for information in this connection the reader may be referred to the paper of Mr. Seymour-Jones already quoted,

FIG. 16.
P. decumbens ON HIDE × 100.

and to an abstract from the German journal "Haute und Lederberichte," in the *Leather Trades' Review*, No. 44, 1921, p. 804.

On tanning materials: It has been isolated from the dust of sumach, valonia and quebracho, in all of which conidia are present in great numbers. In an examination of the green scum from tan liquors it was found to be the dominant organism, and it grows well in laboratory extracts of both valonia and quebracho.

On wort agar: Vigorous growth at 20°C., at first blue-green, then grey-green, and finally green; in old dry cultures, brown. Thom says: "The surface growth consists of trailing or stolon-like hyphæ, sparsely developed, and so close to the substratum as to appear only as fertile hyphæ, bearing the conidiaphores as short branches, 20—100 μ. in length, in old cultures with dense lifts of sterile mycelium scattered upon the surface; conidial fructification consisting of single verticils of crowded conidiiferous cells." Drops of fluid are excreted from the mycelium and lie on the surface of the culture. On this medium it is practically free from odour. Under low powers of the microscope, it is readily observed that this species falls within the section Aspergilloides.

On 15 per cent. gelatine: It develops slowly and not very uniformly, without liquefaction. Cultures on this medium, kept for six weeks at 20°C., show no signs of proteolytic action, and as already noted, this is a distinguishing feature among the four species under discussion.

P. lanosum WESTLING ("Uber die grünen Spezies der Gattung Penicillium," 1911, p. 97, Fig. 60).

Occurence in nature: First described from cultures obtained from specimens found on a species of lichen. In this country its occurrence was first noted in a Petri-dish infection from the atmosphere, and it has since been found on germinating barley, on dead leaves and on leather. Its appearance on such diverse substrata leads to the belief that it is an organism widely distributed in nature.

On leather : It was obtained as a secondary infection in the examination of leather attacked by a black mould, *Aspergillus luchuensis* Inui. It grows in small grey-green colonies, imparting a spotty appearance, and, in early stages, it might be confused with *P. expansum*. It produces long, aerial filaments, however, giving rise to a much more woolly appearance than is exhibited by a colony of the last-mentioned species ; the latter feature is so strongly marked that it becomes a ready means of identification. In the case mentioned, the flourishing condition of the colonies suggested that leather proved a very suitable medium for development, and, although this is the only recorded instance of its occurrence on this material, it may be expected that a species capable of developing on such a substratum, will do so fairly commonly.

On tanning materials : It has not been met with in the dust of those tanning materials so far examined, but it was found to develop slowly, and characteristically, on valonia and quebracho extracts, with a decidedly darkening effect on the liquid. According to Westling, it grows in a 10 per cent. solution of tannin (presumably gallotannic acid) producing only a white mycelial growth. In the writer's experiments, continued for several months, conidia were finally developed with the production of the usual colour.

On wort agar : At 20°C. it grows profusely on this medium, and its most characteristic feature, from the first, is a soft woolly appearance. The surface growth possesses a bluish, or greyish-green colour, and a culture at any stage always contains an admixture of grey filaments which emphasise the woolly appearance. It is not often that a specific name so accurately describes the habit of an organism, as that chosen by Westling for this species. In old cultures, the colour becomes dark brown, but otherwise, the woolly appearance persists. The underside is white or yellowish white. It possesses a very slight musty odour.

MORPHOLOGICAL DATA
(ON WORT AGAR)

	P. expansum.	*P. decumbens.*	*P. lanosum.*	*P. viridicatum.*
Section	Eupenicillum	Aspergilloides	Eupenicillium	Eupenicillium
Conidiaphores ...	Short lateral branches of hyphæ, or long (1–2 mm.) fasciculate coremia ; smooth, 3·6-4·8μ diam.	Short branches, 20–100μ long ; smooth, 1·6–3·6μ diam.	Smooth, 1 mm. long, 3·4–4·6μ diam.	600μ long ; smooth, 4·4–6·5 diam.
Metulæ	3·6–4·5μ × 12–18μ	Absent	3–4·6μ × 12–14μ	4–5·6μ × 10–12μ
Sterigmata ...	2·6–3μ × 7·5–10μ	1·5–2·7μ × 7·5–10·5μ	2–2·7μ × 7·5–9μ	3·2–2·8μ × 7·5–10·5μ
Conidia	Elliptical to globose; smooth, 2 × 3·3μ–3 × 3·4μ	Globose, occasionally oval, smooth, 2·5–3μ	Globose, smooth, or delicately warted, 2·2–3μ	Globose, smooth, 3–3·8μ

On 15 per cent. gelatine : Liquefaction occurs less slowly than with *P. expansum*, but in 14 days, at 20°C., proteolytic action is obvious. Growth is slow, uneven, and characteristic only in patches ; dirty greyish-green in colour.

P. viridicatum WESTLING (*loc. cit.* p. 88, Fig. 56).

Occurrence in nature : This species has been found by Westling on grapes, bananas, alkanet root, and twigs of alder. In this country it has been isolated from decaying fruit, and its conidia have been obtained in Petri-dish examinations of the atmosphere in the neighbourhood of Leeds.

On leather : There is no record of its occurrence on this substratum and it is included here only by reason of its presence in a tan liquor vat.

On tanning materials : It was isolated from a mixture of organisms collected from a tan liquor, consisting mainly of Mycodermæ (including *Mycoderma tannica* Asai), but also containing the present species and *P. decumbens*. In extracts of both valonia and quebracho it developed very slowly, the liquid in each case becoming darker in colour ; a dark brown precipitate was also produced. Westling says, " In tannin solution it forms only a small mycelium."

On wort agar : At 20°C. this species develops rapidly. The colour of the surface growth is green through all its stages ; at first, bright pale green to darker green ; finally, in old cultures, olive green. A growing colony exhibits a white edge as in the case of *P. expansum*, the depth of colour increasing towards the centre of the colony. The texture of the surface growth is floccose, and in this respect again, it is similar to *P. expansum*. The underside is yellow. It possesses a strong disagreeable odour, quite different from that developed by any one of the foregoing species. Crystals of calcium oxalate are freely produced in the substratum.

On 15 per cent. gelatine : At 20°C. liquefaction commences after about 7 days and proceeds until the gelatine is completely liquefied.

F. A. MASON.

The Detection and Estimation of Fluorides

IN the course of research work in these laboratories on the preparation and properties of various fluorine compounds, it became necessary to control certain processes by determining the fluoride content of a large number of solutions. The usual gravimetric method being too laborious and too lengthy for the object in view, for which only moderate accuracy was required, search was made for a rapid volumetric method. It was found that alkali fluorides formed, with ferric chloride, sparingly soluble ferrifluorides, *e.g.*, $6\ KF + FeCl_3 = K_3Fe\ F_6 + 3\ KCl$, and a method was being worked out on the basis of this equation, when it was found that this reaction had already been investigated by Greeff (*Berichte* 1913, 46, 2511).

Greeff dissolves 0·5 grm. of the substance in 25 c.c. of water, adds 20 grm. sodium chloride to diminish the slight solubility of the ferrifluoride, and then 5 c.c. of a 20 per cent. potassium thiocyanate solution and titrates with standard ferric chloride solution until a faint yellow colour is produced. 20 c.c. of a mixture of equal volumes of alcohol and ether are added and the titration is continued, with vigorous shaking in a stoppered flask, until the red colour in the ether layer no longer disappears. The method was tried on a number of soluble fluorides and found quite satisfactory, except that it was considered advisable to standardise the iron solution against a solution of pure fluoride, instead of relying on the stoicheiometrical proportions. For this purpose specially purified potassium fluoride was employed, which was neutral in reaction and free from silicofluoride.

It was found that the method could be somewhat simplified by working with an alcoholic solution of ferric chloride. To 5 or 10 c.c. of the neutral fluoride solution are added 5 c.c. 10 per cent. ammonium thiocyanate solution and 25 c.c. alcohol and the mixture is titrated directly with alcoholic ferric chloride, standardised against pure potassium fluoride in exactly the same manner as that in which the titration is carried out. With a little practice it is possible to obtain readings which do not differ by more than ·05–·10 c.c. It is essential that the solution be neutral and that the same volumes of indicator and solution be always used, as the presence of more water destroys the final colour and thus gives high results. A convenient strength of the ferric chloride solution is between 1 and 3 per cent., but this depends on the strength of the fluoride solution to be analysed. Other substances besides thiocyanates were tried as indicators, but with the exception of sodium salicylate, none proved of use. The colour which ferric chloride gives with phenolic compounds like tannic acid and pyrogallol was not destroyed on the addition of fluoride. An attempt to convert this reaction into an iodometric process was also unsuccessful.

The reaction between ferric chloride and fluorides can be used for the detection of the latter. The solution to be tested is neutralised, if necessary, a little thiocyanate solution is added, and then ferric chloride drop by drop. In the absence of interfering substances, the presence of fluoride is indicated by the rapid disappearance of the ferric thiocyanate colour. Insoluble fluorides like calcium fluoride can be detected in this manner, if the substance be vigorously rubbed down in a mortar, after the addition of a little thiocyanate and a few drops of ferric chloride solution.

Traces of fluoride in neutral aqueous solution can be estimated in the following manner :—To 10 c.c. of the solution is added 0·5 c.c. 10 per cent. ammonium thiocyanate solution and then 0·1 per cent. ferric chloride drop by drop, from a micro-burette, until a pale orange colour is produced. To six test-tubes are then added varying quantities of a dilute standard potassium fluoride solution, so that when the volumes are made up to 10 c.c. and exactly the same amounts

of thiocyanate and ferric chloride solution added to each, the colour observed in the unknown solution falls within this standard range. If necessary, intermediate standards are prepared, in order to match more accurately the unknown colour. The smaller the concentration of fluoride, the more intense will be the colour produced with the same amount of ferric chloride. Thus, fluoride contents of ·005 per cent. to ·0005 per cent. can be determined by preparing a series of test-tubes containing 0·5,1,2,3,4 and 5 c.c. of a ·01 per cent potassium fluoride solution, diluting these to 10 c.c., adding 0·5 c.c. ammonium thiocyanate solution and then 0·2 c.c. of a 0·1 per cent. ferric chloride solution. A gradation of colours is produced, varying from yellow to deep orange-red, which is very suitable for colorimetric comparisons. If a control test be carried out at the same time, it is possible to detect the fluoride in 10 c.c. of a ·0001 per cent. potassium fluoride solution.

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Study of the Causation of "Ropiness" in Worts and Beers

THERE are numerous references to the etiology of "ropiness" to be found in the literature of the fermentation industries. These references are, however, mainly in the nature of suggestions and convey little definite information on the point. Certainly there is a lack of experimental data accompanying each contention and, having in mind the fact that most of the work on this subject was carried out some thirty years ago, it would seem urgent to apply more recently developed methods and conceptions to the solution of the problem.

In previous examinations of beers showing ropiness we have been unable to reproduce and also to maintain that character by means of pure cultures, and our attention has consequently turned to the question of the essential conditions.

The investigation here described was carried out upon a pitching yeast which had been found to produce a ropy and very acid wort after five days' incubation. Microscopical examination showed that an abnormally large number of bacteria were present, and a bacteriological survey was consequently carried out. The mode of procedure was governed by the considerations alluded to in a previous writing,[1] and the yeast autolysate medium was employed in conjunction with ordinary wort agar.

Four distinct types of bacterial colonies developed in the Petri-dish cultures; these were subcultured and the pure cultures examined in connection with the production of ropiness. Preliminary experiments eliminated all but one of the cultures. The remaining culture had predominated in the original plate culture, and resembled an organism we have frequently isolated from beers and tentatively connected with ropiness.

For purposes of reference this bacterium is designated *Bacterium R*, and its cultural and physiological behaviour differs from that described by previous workers for the organisms they have connected with ropiness. The *Bacterium viscosum* of Van Laer, the *pediococcus* of Lindner, and the *pediococcus* of Pasteur are, unfortunately, not minutely described. Although, throughout the present study, a careful search has been made for previously described organisms they have not been observed.

A " slime " produced in a fluid by micro organisms may be described as a distinct mucilage upon the surface or at the bottom of the liquid, the fluid itself being only slightly viscous—a condition somewhat different from the albumen-like, evenly viscid fluid described as " ropy."

In the typical case of ropiness the beer is comparatively clear, and is apparently normal until it is poured from the bottle, when it is then found to be uniformly viscous throughout.

By cultivating *Bacterium R.* in a sterile beer in the absence, and presence of different amounts of oxygen, it has been shown that the character of the mucilage formed was greatly influenced thereby. Flasks of equal size, and containing equal amounts of beer, were sterilised after having been fitted with tubes for the ingress and egress of a gas. The surface area of the fluid may be assumed to have been the same in each flask. The flasks were inoculated with a pure culture of *Bacterium R.* and incubated in the presence of carbon dioxide, hydrogen and air, respectively; the gases having been bubbled through the fluid for fifteen minutes in each case, and the inlet and outlet tubes then sealed with wax.

In the flask containing carbon dioxide a typical ropiness was produced. There was no film and the original slight turbidity of the beer remained unaltered. The fluid became increasingly viscid and stills maintains that peculiarity.

In the flask containing hydrogen similar results were obtained ; perhaps the ropiness was a little less marked, but no material difference was observed.

In the flask to which air had been admitted there were formed a heavy film and dense turbidity of the fluid whilst, at the bottom of

the liquid (under almost anaerobic conditions), a heavy slime was present as a distinct layer.

The amount of air available to the organism during its growth has been roughly controlled by varying the surface area of the fluid medium. This has shown an apparent connection between slime production and lack of oxygen. Liberal æration results in increased film production ; and it is also noted that less mucilage is formed when the film is heaviest.

The condition of ropiness becomes less marked when the sample is left exposed to the air. The admission of air to the CO_2 and H_2 cultures results, after further incubation, in the formation of a film. The film ceases to develop when the air is again replaced by either of the gases.

A condition essential to the production of a clear, typical ropiness is the presence of only a very limited amount of oxygen. When air is only partially excluded a mucilage is formed which may cause a fairly uniform viscosity, but this is always attended by a film and considerable turbidity. If circulation of air is permitted then a copious film is formed with very considerable turbidity, and little, or in some cases, no slime.

Bacterium R. is able to produce a clear ropy wort when grown together with a yeast in air. This may be explained by the replacement of the air by the carbon dioxide produced in the yeast fermentation. Is it not possible that the symbiotic relationship said to exist between a yeast and the " ropy bacteria " may be accounted for in this way ?

This organism has been isolated from the ropy beer and in pure culture, under certain conditions, it produces a typical ropiness. Furthermore, this same organism has been subsequently recovered in pure culture from the beer in which the defect has been reproduced. So that we may say that the " Koch postulates " have been complied with, and that the casual organism has been definitely connected with the disease in this particular instance.

The investigation of the condition in two further samples of ropiness in beers from entirely different sources, adds confirmation to the above remarks, inasmuch as we have obtained a profuse

growth of organisms morphologically and culturally similar to *Bacterium R.* from both of them. Although ropiness was reproduced by using large quantities of the original samples as inoculant, only a slight ropiness was produced by pure cultures of these organisms in the beers or worts at present at our disposal.* A heavy slime and film are produced under aerobic conditions however.

An organism resembling *Bacterium R.* has been frequently observed in small numbers in beers which have not shown ropiness at the time of examination, and in these cases we have not produced ropiness by employing a pure culture. One reason for this may be that certain compounds required in the synthesis of the mucilage have not been present in the media used. At this stage we do not feel justified in expressing a definite opinion as to the influence exerted by different types of wort. *Bacterium R.* grows slightly in a hop infusion, and produces ropiness in the absence or presence of alcohol or glycerol. An exhaustive bio-chemical study of the phenomenon is necessary, together with an investigation of the energy and nutrition requirements of *Bacterium R.* This, it is hoped, may form the subject of subsequent notes.

A second reason is that *Bacterium R.* is apparently one of a group of closely allied species (analogous to the *coliform* group), or as a species may produce strains differing slightly in certain physiological features. In fermented liquors there certainly exists a group of bacteria morphologically indistinguishable from *Bacterium R.*, and in many respects culturally similar, which differ in the character of their growth upon solid media.

This group, including the bacterium under description, apparently corresponds to, or is very nearly allied to, the group of organisms referred to as the " acetic " bacteria. On account of this obvious relationship the writer is led to incorporate certain notes upon the *Acetobacter* with the record of some of the physiological features of *Bacterium R.*

In the classification of bacteria evolved by the Society of American Bacteriologists[2] the acetic acid bacteria are contained in a separate Genus. We reproduce their generic description :—

* Considerable "ropiness" has since been produced.

GENUS 4. *Acetobacter*. FUHRMANN 1905.

SYNONYMS: *Mycoderma* Persoon 1822; *Ulvina* Kuetzing 1837; *Umbine* Naegali 1849; *Bacteriopsis?* Trevisan 1885; *Gliacoccus* Maggi 1886; *Acetomonas* Jensen 1909.

Cells rod-shaped, frequently in chains, non-motile. Cells grow usually upon the surface of alcoholic liquors as obligate aerobes, securing growth energy by the oxidation of alcohol to acetic acid. Also capable of utilizing certain other carbohydrates, as sugar and acetic acid. Elongated, filamentous, club-shaped, swollen and even branched cells may occur as involution forms.

The type species is *Acetobacter aceti* (Thompson 1852) Committee. We shall make use of the generic name *Acetobacter* in the following discussion implying organisms showing the above characteristic features.

OXYGEN RELATIONSHIP.

Although many workers have described members of the acetic acid group of bacteria as obligate aerobes the writer is unable to confirm this. A culture labelled "*Bacterium aceti*" obtained from another laboratory grows well in the absence of oxygen. This characteristic, common to the organisms isolated in the present study, is the only fundamental difference which excludes them from this group to which their other features show them to belong.

Apart from the consideration of surface tension, the decisive factor of film production is the presence of oxygen at the surface of the fluid. In so far as film production is concerned this is, essentially, an aerobic process; but it is here contended that all of the *Acetobacter* need not necessarily grow aerobically as a film. My own experiments lead me to make this statement which gathers weight from the remarks of Brown[3] in reference to his *Bacterium xylinum*:—

> "Here 1·111 gram. of dextrose was decomposed, 0·720 gram. of which was found as gluconic acid, the remainder having gone for the production of cellulose, food for the cells, etc. Yet—notwithstanding active fermentation having been carried on—the total weight of the ferment formed was only about one-fifth of that found when the ferment grew in a solution of lævulose, although no fermentation was apparent in the latter experiment. This seems to raise the question as to whether the "vinegar plant" derives any benefit itself from the oxidising fermentation power it possesses when grown in presence of dextrose, alcohol, etc., and whether this property may not be something entirely unconnected with the necessities of its growth. My experiments appear to show the possibilities of such being the case, and I intend to investigate these actions further."

It is suggested that, although under aerobic conditions the *Acetobacter* have very marked oxidative properties, they also have an additional or alternative process of anaerobic metabolism. It is not surprising that this feature should escape the notice of investigators who have been mainly concerned with the oxidation of alcohol to acetic acid by the film of the "vinegar plant."

Production of Film and Mucilage.

This is a property possessed of several species of *Acetobacter*. Particularly do *A. xylinum Brown*, *A. Pasteurianum* and *A. Kützingianum* produce a mucilage. In short, under certain conditions, they are "slime-forming" bacteria. Yet, in three standard works on this subject we find "Acetic Acid" and "Slime-forming" bacteria treated under separate headings, and one is not led to infer that there may be a definite connection between the two groups. That "slimes" are caused by some species of *Acetobacter* is undisputed. Those organisms connected with ropiness by early workers were very probably distinct species, but from their vague descriptions one cannot say that they do or do not come within the category of *Acetobacter*.

The reaction of the mucilage or film towards the usual tests for cellulose and allied compounds appears to vary considerably with the different species. Chester[4] uses the difference of behaviour of the film with iodine, and iodine and sulphuric acid, as a means of differentiation of species. Brown stated that *A. xylinum* produced cellulose, whilst *A. aceti* gave no tests for that compound.

In the light of our further knowledge of the mucilages and cellulose compounds we know that the blue colouration obtained with iodine and sulphuric acid, or iodine-zinc chloride, and the degree of solubility in cuprammonia is variable within each group.[5] By hydrolysis celluloses, starches and the mucilages may all yield glucose. So that conclusive differentiation between these compounds cannot really be made by means of above reactions.

The mucilage produced by *A. Pasteurianum* and *A. Kützingianum* is stated to give blue colour reactions with iodine. The film and mucilage produced by *Bacterium R.* has not, so far, given us the blue colouration, and it does not appear to be soluble in cuprammonia

(Schweitzer's reagent). The mucilage is easily separable from the ropy fluid by the addition of excess alcohol or acetone. A sticky, glutinous mass is obtained which gives reactions typical of the series of more complex gums and mucilages than of cellulose; in appearance it resembles the protein Glutin. We are cultivating the organism in bulk and hope to produce a sufficiently large quantity of mucilage for the series of determinations indicated.

Brown,[3] describing the growth of *Bact. xylinum* says :—

> "If the 'vinegar plant' is grown in a liquid unfavourable to its free growth, such as yeast-water, it is first observed as a jelly-like, very transparent mass at the bottom of the solution; and this gradually increases in size until the surface is reached, and the liquid appears to be almost entirely filled with it. Close examination of this mass, however, shows that it is composed of extremely attenuated membranes of the ferment, which ramify through the bulk of the liquid and actually enclose much of it, thus giving the jelly-like appearance."

We have been much inclined to identify *Bacterium R.* with *Acetobacter xylinum* Brown, but several observations made by Brown in connection with the latter, chiefly with regard to the production of cellulose, do not permit us to do so.

Involution Forms.

Hansen demonstrated the influence of temperature upon the pleomorphism of *Bact. aceti.* and showed that at about 40°C. the long, swollen, irregularly-shaped involution forms so characteristic of the *Acetobacter* were most abundantly produced. Brown[3] states that *A. xylinum* produced involution forms in a medium unfavourable to growth, but not those of the swollen type. He does not mention the influence of temperature. The behaviour of *Bacterium R.* in this connection is as follows :—

Growth upon yeast autolysate medium—

At 10°C., poor : involution forms very few in number and short.
,, 20°C., moderate : involution forms rare and short.
,, 27°C., good : involution forms not found.
,, 35°C., fairly good : many very long involution forms including types described by Hansen as typical of *Bact. aceti.*
,, 40°C., no growth : inoculant not involuted.

Only rare, short involution forms were found in old cultures (20–27°C.) in wort, beers, glucose-yeast water and in the anaerobic cultures. Our culture of *A. aceti* behaves similarly in this last respect.

Morphological and Physiological Features of *Bacterium R.* (*? Acetobacter R*).

Growth upon yeast medium, 24–48 hours, at 27° C. :

Short, stumpy rod, $0{\cdot}7\mu \times 1{\cdot}2\mu$ (average), variable from coccus-like to distinct rods, many forms resembling diplococci (" hour-glass " forms noted by several workers). Mainly in pairs. Some cells occurring singly, but none as chains or filaments at this stage. After some days' incubation short chains and somewhat elongated rod forms may develop. Gram negative. No endospores. Motility not evident.

Colonies upon yeast medium or wort agar :

Large, round, pulvinate, entire, smooth, glistening, translucent becoming cloudy, viscid.

Colonies on gelatin media :

Smaller, convex, not so moist, less viscid, denser.

Slope cultures—Yeast medium :

Filiform, translucent at first, developing dense cloudiness later ; slimy.

Upon gelatin media a drier growth is obtained which becomes centrally dense with hyaline edge.

Optimum temperature 25–30°C. *Indol* not positive.

Voges-Proskauer reaction negative. Gelatin and starch not liquefied. Milk not changed. Nitrates appear to be reduced, but this is difficult to ascertain owing to the slight growth obtained in media suitable to the test.

Facultative anaerobe :

Growing equally well in hydrogen or carbon dioxide alone, in air, and in air from which oxygen has been removed (Buchner tube with alkaline pyrogallol).

Film production is influenced by the factor of æration alluded to above. The character of the film varies between dense, almost brittle, and more or less translucent, gelatinous or slimy. Reddish-brown colouration obtained with iodine or iodine and sulphuric acid.

Preliminary fermentation tests give the following results :

No change in dextrin, starch, raffinose, lactose, maltose, sucrose, lævulose, galactose.

Acid and no gas in glucose, alcohol, and (?) acid in glycerol.

Acidity reduced in acetic acid solution.

It will be seen that these reactions are typical of the *Acetobacter*

An illustration appearing in " Etudes sur la bière," Pasteur,[6] 1876, is of interest in view of the above. Drawings of the causal organisms of seven diseases of beer are shown upon one plate. It is remarkable that the drawing of the organisms from ropy beer and that of the acetic ferment, " mycoderma " *aceti*, resemble each other so closely whilst the remaining five organisms have no feature in common. At that date, of course, Pasteur would not be aware of the pleomorphic tendency of the *Acetobacter*. We now know that chains of practically spherical cells may occur in addition to the other forms, and this might be reasonably held to account for the slight difference between the two organisms of Pasteur's drawings.

NOTE.—In view of the lack of conformity of opinion regarding ropiness, the above investigation was carried out as an independent study. It is now our object to correlate these findings with those of other workers, in particular Baker, Day and Hulton, whose results, in part, are corroborated.

Some experimental data are for the present omitted in order that they may appear together with others now in the course of preparation.

P. HAMPSHIRE.

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"Speckled" Malt

UNDER the name of "speckled" malt, brewers are familiar with certain discoloured grains found more or less frequently in malted barley. Owing to the peculiar blotchy or often black-banded appearance presented by these grains, they are also commonly known as "magpies."

According to the theory advanced by the late W. Frew (*Journal Soc. Chem. Ind.*, 1898, 17, p. 330), the charring of the husk is due to the presence of free sulphuric acid produced by the oxidation of sulphurous acid which has been absorbed by the moist husks from the kiln gases. This condition is believed to be brought about by defective draught arrangements, and in Frew's experience "speckling" did not occur on the old-fashioned single-floor kiln, but only on double kilns having iron tiles on the lower floor. It was further stated that the liability to produce such grains is greater when coal is used for fuel than is the case when coke is used.

A sample of malt recently received for analysis contained a much larger number of "magpies" than usual, these being present to the extent of 10 per cent. of the total grains. Such a sample would be regarded by the brewer with grave suspicion, although there is a dearth of information with regard to the specific internal differences between "magpies" and normal grains. In the absence of this information it appeared desirable to obtain some experimental evidence of the influence of "magpies" on the brewing value of the sample. Unfortunately, the stripping did not represent an ideal

malt for the purpose as it had been dried off at too high a temperature, which resulted in the production of much colour, together with a crippling effect on the diastase; otherwise, the large percentage of " magpies " rendered it suitable material for separation.

As opposed to Frew's observations this malt had been cured on a single, pot-tile floor, fired with coke. The maltster informs us that he usually gets a few " magpies " on this kiln, and that the proportion varies with different consignments of coke. The fact that those consignments which tend to produce much smoke also produce the greater proportion of " magpies " appears to confirm Frew's opinion with regard to fuel. The kiln had just undergone alteration for ventilation, and proper adjustment of draught had still to be made, hence the results which exhibit lack of temperature control.

The figures obtained on the sample, as received, are as follows :

Moisture...	0·30 per cent.
Brewers' Extract	98·78 lbs. per bar. per qr.
Tint (Lovibond, 1″ cell) ...	22°
Specific Rotation	122·5°
Diastatic Power (Lintner) ...	14°
Matters soluble in cold water	19·94 per cent.

From a large sample of this malt, " magpies " were separated in sufficient quantity to carry out parallel estimations on these and the normal grains, using the usual quantities of materials according to the methods of the Malt Analysis Committee.

If free sulphuric acid were present in the blackened grains, or indeed more acid of any kind than is present in normal grains, the colorimetric method of determining hydrogen ion concentration would infallibly demonstrate the fact. Two portions consisting respectively of 20 " magpies " and 20 normal grains, were each placed in a test tube, covered with 10 c.c. of distilled water, and

allowed to stand at 20°C. for 12 hours, after which, the reaction of the two solutions was found to be identical, giving pH 4·5 for each of them. This figure is normal for malt, and incidentally, it is the optimum pH value for yeast activity.

An analysis of the separated grains gave the following results :

	Normal Grains.	"Speckled" Grains.
Brewers' Extract	96·8 lbs.	97·1 lbs.
Tint (Lovibond 1" cell) ...	20°	26°
Specific Rotation	122·5°	122·8°
Diastatic Power (Lintner) ...	14°	14°
Acidity—		
Litmus	7 c.cs.	8 c.cs.
Phenol phthalein	20 c.cs.	22·2 c.cs.

In determining the acidity, titration was carried out in two stages, using Litmus and Phenol phthalein as indicators, according to the method of A. Reichard (Zeit. ges. Brawesen, 1916, through the *Journ. Inst. Brew.*, 1917, 23, p. 117). Reichard has shown that the litmus acidity represents the more strongly acid substances of the grain, including tannic acid and other acids present in the husks, in addition to lactic acid ; the phenol phthalein acidity represents more feebly acid substances such as acid phosphates and some amino-acids (also *Journ. Inst. Brew.*, 1919, 25, p. 76). The figures were obtained on a 10 per cent. solution of ground malt digested at 21°C. for three hours, and the results are expressed as c.cs. of N/2 NaOH per 100 grams. malt. Although the figures obtained on the two samples exhibit differences which indicate a higher acidity in the case of the " speckled " grains, it cannot be said that they contain more free acid than is found in normal strippings, and indeed, so far as this particular sample is concerned, the acidity is below average. In practice, normal samples of malt not containing " magpies," are found to give figures varying from 4·0 to 16·5 c.c. with litmus, and between 18·0 and 29·4 c.cs. with phenol phthalein ; mean values

obtained in the case of a series of twelve samples recently examined giving for litmus 12·6 c.cs., and for phenol phthalein 24·4 c.cs..

Since the views of Frew were published we have been made aware of the existence of a series of dark-coloured, tinctorial substances, produced at high temperatures by the action of amino-acids on carbohydrates. These bodies, termed Melanoidines by Maillard, who first described them, are produced under the conditions of kilning, and their occurrence in roasted malt has been investigated by Ruckdeschel (*American Brewers' Journal*, 1915, p. 107). We would suggest that the "speckling" of the grains in the sample examined by us is due to the production of one or more of these melanoidines in the outer cells of the husks, brought about by some unknown conditions of moisture content and the nature of the kiln gases, in relationship to ventilation and temperature. Microscopical examination of the blackened husks indicates the production of colour only in the outer cells, each of which contains a spherical, semi-transparent brown particle, unlike anything seen in the normal portions of the husk. This suggests the formation of compounds *inside* the cell rather than mere charring of the cell structure.

The extract and specific rotation figures of the "speckled" and normal grains are in such close agreement that it seems unlikely that the endosperm contents of the two portions differ in any way. The lower extract values of these two samples, as compared with the original, are due to the increase of moisture consequent upon exposure during the long operation of separating the grain by hand. The only variable feature is the deeper colour of the wort obtained from the "speckled" malt, and this may reasonably be attributed to the extra colouring matter in the husks.

From these experiments it may be concluded that except for an increase of tint, the brewing value of a malt remains unimpaired by the presence of "speckled" grains.

F. A. Mason and F. Brown.

A Note on "Spued" Leathers

WE have recently examined a number of "spued" leathers, and boots which had developed spue after having been worn for a short time. Considerable trouble has been caused to manufacturers and retailers of the present time owing to the prevalence of complaints of "spued" goods. There are several different types and causes of spues* and those occurring most commonly may be classified as follows :—

"Fat Spues" :—1. Due to the decomposition of fats in the leather into fatty acids, etc., either by direct chemical oxidation, or by lipases produced by micro-organisms.

The deposit of fatty acids may be resinous and sticky, or it may be white, powdery and dry ; this will be decided by the nature of the fats used in currying. Occasionally both the white and the resinous types may occur simultaneously.

2. Due to crystallisation of hard fats used in currying.

Fat spues temporarily disappear on warming the leather, but they are only entirely removable by the use of solvents.

"Damp Spues" :—Usually caused by deposition of the fat-liquor upon the grain of imperfectly dried goods, or goods which have been stored in a damp place.

When this spue is once wiped off it is not, as a rule, recurrent.

* A white, powdery growth of mould is often mistaken for a "spue," and *vice versa*.

"GLUCOSE SPUES":—May occur upon leathers with high glucose content.

"SALT SPUES":—We coin this term to indicate so-called spues which are, in reality, depositions of inorganic salts upon the surface of leathers. The weighting of the leather, or fabric lining of footwear, with magnesium sulphate, etc., has frequently been proved responsible for this type of spue. It is particularly detrimental to footwear, but may usually be dusted off the surface of leather before it is made up.

When this type of spue occurs upon worn boots or shoes a more or less heavy incrustation occurs at points corresponding to the working joints of the foot.

In the examples we have recently studied the cause of the spue has been other than that referred to above. In each case the leather was mineral tanned—chrome or semi-chrome; the deposit was apparently sodium sulphate. This salt presumably remained in the leather after neutralization, and it is significant to note that the prevalence of this class of spue has increased with the growth of popularity of chrome-tanned upper leathers.

We have employed a rough, though rapid, method of demonstrating this spue. A cutting from the affected part of the shoe is placed flesh downwards upon a piece of moistened cotton-wool; spues of this type then disappear almost immediately, and at points where the deposit is heaviest a bead of moisture forms.

In the case of one sample a cutting from an unaffected part of a damaged shoe was extracted with water, the extract was concentrated, and applied to the surface of a sound leather. When dry this leather showed a spue, although moistening the leather with

water alone, or an extract of the sound leather, did not produce this effect.

A piece of semi-chrome box calf showing a spue of inorganic origin, after having been dusted free of the deposit, was wetted unevenly and again dried. The spue recurred most heavily in those places which had been wettest.

It is suggested that the parts of the shoe in juxtaposition to the working joints of the foot are moistened by perspiration at a particular point, and moisture is absorbed into the surrounding leather. Drying proceeds in the reverse direction, and a central focus of the moisture is formed at the original point of contact. In this way the leather over the joint is extracted of its soluble compounds which are concentrated at those points receiving most perspiration. This concentration, subsequent crystallisation and further wear of the shoe result in very evident damage to the leather. Microscopical examination shows a more or less amorphous surface deposit and crystals disposed in the midst of the fibres in the interior of the leather. Marked injury to the fibres is very apparent and is obviously due to mechanical abrasion of the crystals.

The writer recently purchased a pair of brown shoes of a highly reputed make. Upon complaining to the retailer in respect of the salt spue which appeared after two days' wear, that gentleman informed us that it was merely "the goodness coming out"—presumably there in excess. This, of course, might have been a half-truth had the trouble been a fat spue, but it would be better if this kind of "goodness" could be removed by washing after the process of neutralization of the leather.

An effective remedy has been to wipe over the affected part of the shoe at frequent intervals with a moistened cloth.

P. Hampshire.

Our Agricultural Testing Station

SOME NOTES ON PRESENT CONDITIONS AND THE WORK OF 1921-22

OWING to the exceptionally light and open nature of our soil, difficulty was experienced in maintaining the various crops in a healthy growing condition during such an abnormal season, and, owing to the lack of potash, the majority of the crops lacked the stamina and robustness this necessary element provides.

The Station included a Hop Plantation used for spraying experiments, one of our principal tests for all Insecticides and Fungicides being, that for Summer use, the material shall not damage the foliage of this tender plant. *Aphis humuli* was the chief pest, with isolated cases of Red Spider, *Tetranychus telarius.*

The nursery contains a selection of Paradise stocks obtained from the East Malling Experiment Station. These were quickly attacked by Wooly Aphis, *Schizoneura lanigera,* which gave us opportunities for testing Alvesco Specialities. *S. lanigera* is an extremely difficult pest to control on trees in the neighbourhood of elms, upon which certain stages in the life-history of this aphis are passed, and the nursery is surrounded by elms. Experiments are in progress and these promise well.

Red Spider is one of our most serious pests. Runner Beans, Violets, Carnations, Cucumbers and Parsnips are all attacked, and, throughout the year, work has been in progress with Insecticides compounded for dealing with this pernicious pest. Notes on control of the Red Spider were published in *Bulletin* No. 5, p. 143, and the methods outlined therein were found satisfactory here.

Cut Worms are represented by the larvæ of the Heart and Dart Moth, *Agriotes exclamationis,* causing damage to *Brassicæ,* Beet, and Carrots ; these are pests presenting serious difficulties, both commercial and practical, in regard to their elimination, but methods for their control are receiving attention.

The Onion Fly, *Anthomyia ceparium*, and Carrot Fly, *Psila rosea*, were effectively controlled by the use of Insecticides containing various tar oils and Chlor-cresol compounds.

Various soil sterilization experiments were carried out on Potato plots, results of which it is hoped to publish later ; reference to such experiments, in connection with Tomato growing, has already been made in *Bulletin* No. 5, p. 134.

A number of Apple trees have been attacked by Powdery Mildew, *Sphærotheca mali ;* this fungus has been controlled by the use of Ammonium Polysulphide.

A Mushroom bed was found to be badly attacked by mites, which were readily eliminated by the use of our White Fly Fumigant.

Considerable success has attended our experiments for the extermination of White Fly, *Asterochiton* (*aleyrodes*) *vaporariorum*, with tetrachlorethane and other chlorine compounds.

So far, most of the experiments can only be considered of a preliminary nature, and they are being continued. Circulars containing particulars of all Alvesco Agricultural and Horticultural products at present on the market may be obtained upon application to Murphy & Son, Ltd., Cedars Laboratories, Sheen Lane, Mortlake, S.W. 14.

J. S. Mitchell.

The Cedars Laboratories, Mortlake, S.W.

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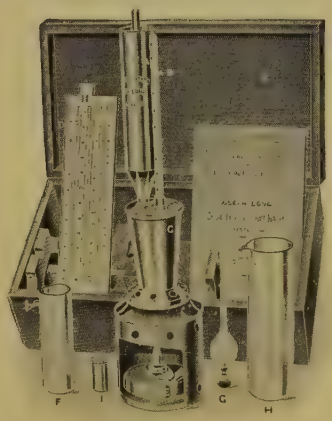

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